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Dissociation of urate from sodium transport in the rat proximal tubule

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Dissociation of urate from sodium transport in the rat proximal tubule. The tubular transport of urate and sodium was examined by clearance, free-flow micropuncture, intratubular microinjection and precession techniques in control rats and in rats receiving a new uricosuric diuretic, indanyloxyacetic acid (MK-196). The i.v. infusion of MK-196 (50 mg/kg of body wt/hr) resulted in significant increases in the fractional excretion of sodium (FE_{Na}) from 0.98 ± 0.01 to 11.86 ± 2.88% ($P < 0.001$) and in FE_{urate} from 14.1 ± 1.03 to 56.0 ± 2.86% ($P < 0.001$). End-proximal tubular fluid to plasma inulin (TF/P_{inulin}) ratios were 2.43 ± 0.15 and 2.51 ± 0.10 in control and drug-treated animals, respectively (P = NS). Total urinary urate recovery after MK-196 administration was higher following microinjections of [2-^{14}C] urate into early proximal tubule sites: 70.5 ± 2.7% in controls vs. 84.9 ± 0.9 ($P < 0.001$), and after microinjections into late proximal tubule sites: 82.8 ± 2.9% vs. 91.3 ± 1.9 ($P < 0.05$). Urinary precession of urate from inulin was demonstrable following placement of isotopes of these compounds on the surface of the kidney in controls, but was abolished by MK-196. This agent, therefore, inhibits the reabsorption and secretion of urate in the proximal convoluted tubule, the net effect being a marked increase in urinary urate excretion. By contrast, its inhibitory effect on sodium reabsorption is exerted at a site or sites distal to the accessible portion of the proximal tubule. The demonstration of reduced urate reabsorption and normal sodium reabsorption in the proximal tubule suggests that the reabsorption of these constituents of the glomerular filtrate is not intimately linked at this nephron site.

Dissociation du transport de l'urate de celui du sodium dans le tube proximal de rat. Le transport tubulaire de l'urate et du sodium a été étudié par clearance, microponctions, microinjections intra-tubulaires et recherche de précession chez des rats contrôles et des rats recevant un nouveau diurétique uricosurique, l'acide indanyloxyacétique (MK-196). L'administration intraveineuse de MK-196 (50 mg/kg poids corporel/heure) détermine une augmentation significative de FE_{Na} de 0,98 ± 0,01 à 11,86 ± 2,88% ($P <$ 0,001) et de FE_{urate} de 14,1 ± 1,03 à 56,0 ± 2,86% ($P < 0,001$). Le rapport de concentration de l'inuline à la fin du tube proximal est de 2,43 ± 0,15 et 2,51 ± 0,10 chez les contrôles et les animaux traités respectivement (P = NS). La récupération urinaire des microinjections d'urate [2-^{14}C] dans les tubes proximaux précoces est plus grande après MK-196: 70,5 ± 2,7% chez les contrôles, 84,9 ± 0,9% après MK-196 ($P < 0,001$). Elle est plus grande aussi après microinjections dans les tubes proximaux tardifs: 82,8 ± 2,9 et 91,3 ± 1,9% ($P < 0,05$). La précession urinaire de l'urate sur l'inuline a été démontrée après dépôt des isotopes de ces substances sur la surface des reins des contrôles. La précession est abolie par MK-196. Ce corps, par conséquent, inhibe la réabsorption et la secretion d'urate dans le tube contourné proximal, l'effet net est une augmentation importante de l'excrétion urinaire. Son effet inhibiteur sur la réabsorption du sodium est exercé, au contraire, sur un ou des sites situés en aval du tube proximal accessible. La mise en évidence, dans le tube proximal, de la diminution de réabsorption d'urate et d'une réabsorption non modifiée du sodium suggère que les réabsorptions de ces constituants du filtrat glomérulaire ne sont pas étroitement liées dans cette région du néphron.

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A number of recent studies have contributed to the understanding of the bidirectional transport of urate [1–11]. Prior studies from this and other laboratories have indicated that, in the rat, urate reabsorption occurs primarily in the proximal convoluted portion of the nephron and that the tubular reabsorption of sodium and of urate can be altered independently [1–3,9]. Preliminary reports have indicated that a new compound, indanyloxyacetic acid (MK–196), is a potent diuretic and uricosuric agent [12]. It was of interest, therefore, to determine the tubular site or sites of altered urate and sodium transport induced by this agent. In the present studies, clearance, free-flow micropuncture, microinjection and precession techniques were employed to examine the renal transport of urate and of sodium following the administration of MK-196.

Methods

Male Sprague-Dawley rats weighing 200 to 400 g were used in all experiments. In the micropuncture, microinjection and precession studies, anesthesia was induced by the i.p. injection of Inactin (Promonta, Hamburg, Germany), 100 mg/kg of body wt. After a tracheostomy, both jugular veins, a femoral artery and vein and the urinary bladder were catheterized. The left kidney was decapsulated and prepared for micropuncture as previously described [13]. Estimated surgical losses of fluid were replaced with a volume of isotonic saline equal to 1% of body wt. In the microinjection and precession studies, the left ureter was cannulated near the renal pelvis to permit separate urine collections from each kidney. MK-196 was dissolved in distilled water containing an equal

concentration of sodium bicarbonate and delivered in a dose of 50 mg/kg of body wt/hr in a volume calculated to deliver 1.2 ml/hr. Control animals received the same solution without the drug.

Free-flow micropuncture studies. After surgical preparation, six control and eight experimental animals received 100 μCi of [methoxy-^{3}H]-inulin in 1 ml of isotonic saline, infused as a prime, followed by the infusion of the same solution at a rate of 1.2 ml/hr. Urinary losses of sodium were replaced with a volume of isotonic saline equal to the urine flow rate. After 60 to 90 min, when the urine flow rates were stable, micropuncture samples were obtained from end-proximal tubule sites as previously described from this laboratory [13]. Simultaneous timed urine collections were obtained in two or three 30-min intervals. One milliliter of arterial blood was obtained from the femoral artery at the midpoint of each period and was replaced with the same volume of blood from a donor rat. Hematocrit values were recorded prior to and during the infusion of the diuretic. At the completion of the study, the kidneys were removed, stripped of perirenal fat and capsule and weighed in an analytical balance.

Intratubular microinjection studies. Eight control and nine experimental animals were prepared for study as indicated above, except that inulin was not infused i.v. An infusion of 5% mannitol in isotonic saline was administered at a rate of 12.5 ml/hr to insure high urine flow rates. The urine flow rate of the micropunctured kidney was 85% or higher than that of the contralateral kidney. Urinary losses of sodium and water due to the diuretic were not replaced. Intratubular microinjections were performed with a solution containing [2-^{14}C]-urate (50 μCi/ml) and [methoxy-^{3}H]-inulin (100 μCi/ml) adjusted to a pH of 7.4 with a solution of $NaHCO_3$ (30 mEq/liter). The final urate concentration was 4 mg/100 ml. Triplicate droplets of 12 to 20 nl were prepared, one of which was utilized for the microinjection and the other two counted directly for total radioactivity. Microinjections were performed into early or late proximal tubular sites over a 60- to 90-sec interval and total urine collections from both left and right kidneys obtained at 30-sec intervals for 10 to 15 min. The procedures for microinjection, the localization of injection sites and the calculations of the recovery rates were identical to those of Kramp, Lassiter and Gottschalk and have been described in detail from this laboratory previously [1, 9].

Urate precession studies. The urinary precession of urate from inulin was utilized as an index of urate secretion. Five control and five experimental animals were prepared for study as in the microinjection studies except that 5% mannitol in isotonic saline was infused at a rate of 22.5 ml/hour. One hundred nanoliters of the [2-^{14}C]-urate and [methoxy-^{3}H]-inulin solution was placed upon the surface of the decapsulated left kidney as a droplet. Urine was collected in 15-sec aliquots from the left kidney only. No attempt was made to quantitate total recoveries. The criteria for precession of urate from inulin were (*1*) an arrival time of [2-^{14}C]-urate in the urine 30 or more seconds ahead of [methoxy-^{3}H]-inulin with the absence of significant inulin counts in the first urine sample containing significant concentrations of [2-^{14}C]-urate and (*2*) an increase in the ratio of [2-^{14}C]-urate/[methoxy-^{3}H]-inulin in the first urine sample to contain significant counts of [methoxy-^{3}H]-inulin compared to the ratio of counts in the droplet solution.

Free water clearance and reabsorption studies. The animals were lightly anesthetized with ether for placement of catheters. An infusion of [methoxy-^{3}H]-inulin (25 μCi/ml) was infused at a rate of 0.02 ml/hr and continued throughout the study. The animals were then placed in restraining cages and allowed to awaken. Free water reabsorption (T^cH_2O) studies were performed in four control and four experimental animals deprived of food and water for 24 hr prior to study. One hour prior to study, 0.5 U of pitressin tannate in oil was administered subcutaneously. After surgical preparation, an infusion of 2% saline was begun at a rate of 0.1 ml/min and allowed to equilibrate 60 to 90 min, following which blood and urine sample collections were started. The infusion rate of 2% saline was then gradually increased so that the infusion rate always exceeded the urine flow rate. Blood and timed urine collections were obtained at each level of infusion rate.

Free water clearance (C_{H_2O}) studies were performed in four control and four experimental animals deprived of food for 24 hr prior to study but allowed free access to a 2% dextrose drinking solution. On the day of study, three doses of a solution containing 1.67% glucose, 1.3% ethanol and 0.13% sodium chloride were given by gavage in amounts equivalent to 3% of body wt, at 20-min intervals. After surgical preparation, the water diuresis was sustained by the infusion of hypotonic saline (0.225%) at a rate of 0.1 ml/min. The infusion rate was then increased in stepwise fashion up to 0.5 ml/min with urine and blood collections obtained at each level of infusion.

Analytic methods. Radioactivity of blood, urine, microinjection and droplet samples was determined in a modified Bray's solution in a liquid scintillation counter (Tri-Carb, Packard Instruments Co., Downers Grove, IL) with appropriate corrections for crossover of ^{14}C counts appearing in the ^{3}H channel.

Clearances, fractional excretions, C_{H_2O} and T^cH_2O were calculated from standard formulae. Hematocrit values were measured in microhematocrit tubes and Na^+ and K^+ by flame photometry. Uric acid in blood and urine was determined by the uricase method using the polarographic oxygen sensor in a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA) [1]. Osmolality was determined by freezing point depression in an osmometer (Advanced Instruments); pH was determined in a pH meter (Beckman). All data are expressed as the mean ± SEM. Statistical significance was determined by either Student's *t* test or Fisher's *t* test where appropriate.

Results

Free-flow micropuncture studies. Following the infusion of MK-196, there was a delayed onset of the diuresis and natriuresis. The onset of the response varied from animal to animal but averaged 30 min, with a peak response at 60 to 90 min. Mean arterial pressure was unchanged following the administration of the drug. Hematocrit values recorded prior to (49.6 ± 0.8%) and during the infusion of the diuretic (50.5 ± 0.6%) were not significantly different, indicating the adequacy of volume replacement. The results of the clearance and micropuncture studies are summarized in Table 1. The administration of the diuretic resulted in significantly higher rates of urine flow: 134.6 ± 18.11 as compared to 8.8 ± 2.06 μl/min in controls; of urinary excretion of sodium: 11.86 ± 2.28 as compared to 0.11 ± 0.02 μEq/min ($P < 0.001$); and of urinary excretion of potassium: 5.16 ± 0.36 as compared to 0.84 ± 0.22 μEq/min ($P < 0.001$). The glomerular filtration rate was slightly lower in the drug-treated animals, but this change failed to reach statistical significance. Of particular interest was the strikingly higher urate clearance of 477.2 ± 53.4 μl/min/g of kidney wt in the drug-treated animals as compared to 141.6 ± 11.3 in controls ($P < 0.001$). The fractional excretion of urate was increased, therefore, from 14.1 ± 1.03% to 56.0 ± 2.86 ($P < 0.001$). The tubular fluid to plasma inulin (TF/P_{Inulin}) ratios of samples obtained from end-proximal tubule sites were 2.43 ± 0.15 and 2.51 ± 0.10 (P = NS) in controls and drug-treated animals, respectively.

Microinjection studies. In order to localize the nephron site of altered urate reabsorption, intratubular microinjections were performed into early and late proximal tubule sites. The urine flow rate in the left kidney averaged 62.2 ± 14.7 μl/min in control animals compared to 132.5 ± 14.9 ($P < 0.02$) following the infusion of MK-196. Only samples in which the inulin recoveries were 95% or greater were included for analysis. No ^{14}C counts were recovered from the right kidney in any animal. Delayed recoveries ranged from 0 to 8% in control and experimental animals with no significant differences between the two groups. The results, therefore, are expressed as total urate recoveries and are summarized in Fig. 1. Compared to controls, urate recoveries were significantly higher in the animals receiving MK-196: 84.9 ± 0.9 ($N = 12$) as compared to 70.5 ± 2.7% ($N = 11$) ($P < 0.001$) after microinjections into the early portion of the proximal tubule, and 91.3 ± 1.9 ($N = 9$) as compared to 82.8 ± 2.9% ($N = 12$) ($P < 0.005$) after microinjection into the late proximal tubule. Microinjections into distal tubules were not performed since prior studies have demonstrated little or no urate reabsorption at these tubular sites [1,3,7].

Precession studies (Table 2). In control animals, radioactive-labeled urate preceded inulin in the urine by an average of 39 sec ($P < 0.001$). The ratio of $^{14}C/^3H$ counts in the first urine sample to contain significant ^3H counts compared to the ratio of counts in the droplet solution was 1.65 ± 0.18 ($P < 0.001$). Following the administration of MK-196, the arrival time of [methoxy-^{3}H]-inulin was prolonged, presumably due to an increase in tubular transit time; the arrival time of urate was not significantly different from that of inulin and the ratio of $^{14}C/^3H$ counts was less than unity.

Table 1. Free-flow micropuncture studies[a]

	Control	Experimental	*P*
Urine volume, *μl/min*	8.8 ± 2.06	134.6 ± 18.11	<0.001
$U_{Na}V$, *μEq/min*	0.11 ± 0.017	16.5 ± 2.42	<0.001
FE_{Na}, %	0.98 ± 0.014	11.86 ± 2.28	<0.001
U_KV, *μEq/min*	0.84 ± 0.22	5.16 ± 0.36	<0.001
GFR, *μl/min/g of kidney wt*	1004.0 ± 66.6	943.0 ± 81.0	NS
C_{urate}, *μl/min/g of kidney wt*	141.6 ± 11.3	477.2 ± 53.4	<0.001
FE_{urate}, %	14.1 ± 1.03	56.0 ± 2.86	<0.001
Plasma urate, *mg/100 ml*	1.43 ± 0.06	1.59 ± 0.13	NS
TF/P end-proximal	2.43 ± 0.15 (29)	2.51 ± 0.10 (25)	NS
Single nephron GFR, *nl/min/g of kidney wt*	29.2 ± 1.34 (29)	28.9 ± 2.61 (21)	NS

[a] Values represent mean ± SEM. Numbers in parentheses indicate the number of observations.

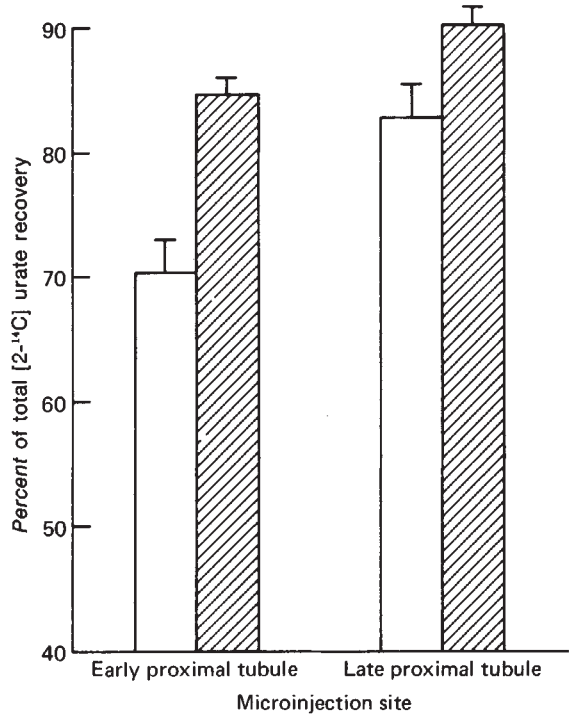

Fig. 1. *Percent of total [2-^{14}C]-urate recovered following microinjections in early and late proximal tubule sites in control* (open bars) *and experimental* (hatched bars) *animals.* Values represent mean ± SEM.

Free water clearance and reabsorption studies (Figs. 2 and 3). In order to further localize the site of impaired sodium reabsorption, C_{H_2O} and T^cH_2O studies were performed in awake animals. As shown in Figs. 2 and 3, fractional free water clearance as a function of fractional urine flow and fractional free water reabsorption as a function of fractional osmolar clearance were significantly reduced following the administration of MK-196 as compared to control values.

Discussion

The results of the present investigations provide further insight into the understanding of the tubular handling of urate, and localize the sites of action of a new diuretic agent, MK-196. Prior studies from this and other laboratories have indicated that urate reabsorption in the rat occurs primarily in the proximal convoluted tubule and that this reabsorptive process is not saturated even if the urate concentration within the tubular lumen is increased well beyond the physiologic range [1–3,4,9]. Although the renal excretion of urate parallels that of sodium in response to most physiologic stimuli, Weinman et al and Roch-Ramel et al have advanced evidence that these constituents of the glomerular filtrate do not share a common transport mechanism [2,3]. Evidence for urate secretion from peritubular blood into the tubular lumen has also been presented, although the magnitude and exact site within the nephron of this transport process remains undetermined [3,4,8,10,11].

The present studies revealed a striking increase in urate excretion following MK-196 administration to values averaging 56% of the filtered load excreted into the urine. This value is higher than those observed in this laboratory following expansion of the extracellular fluid volume with isotonic saline solution [1]. The microinjection technique has been shown to be a valid method of determining the nephron site of urate efflux from the tubule and has the additional advantage of permitting the diuretic to gain access both to the peritubular and luminal sides of the renal cells [1,9]. This was deemed important since the exact locus of action of MK-196 has not been determined. As demonstrated from the microinjection studies, the nephron site of reduced urate reabsorption is the proximal convoluted tubule. The finding of a reduction in urate reabsorption in the proximal tubule is of particular interest since the reabsorption of sodium and water at this nephron site is unchanged from control following MK-196 administration. The observed diuresis and natriuresis are a consequence of altered reabsorption at a site beyond the end of the accessible portion of the proximal tubule and the results of the C_{H_2O} and T^cH_2O studies suggest a major site of action in the ascending limb of the loop of Henle [14]. Thus, there is a dissociation between sodium and urate reabsorption in the proximal convoluted tubule. The findings of a decrease in urate transport in the presence of normal water reabsorption in this segment of the nephron following MK-

Table 2. Precession studies

	Arrival times			
	^{14}C-urate *sec*	^{3}H-inulin *sec*	^{14}C/^{3}H-urine droplet	
Control (N = 14)[a]	94 ± 10	133 ± 4	1.65 ± 0.18	
	$P < 0.001$			$P < 0.001$
Experimental (N = 13)	155 ± 10	164 ± 10	0.86 ± 0.07	
	P = NS			

[a] N = number of observations.

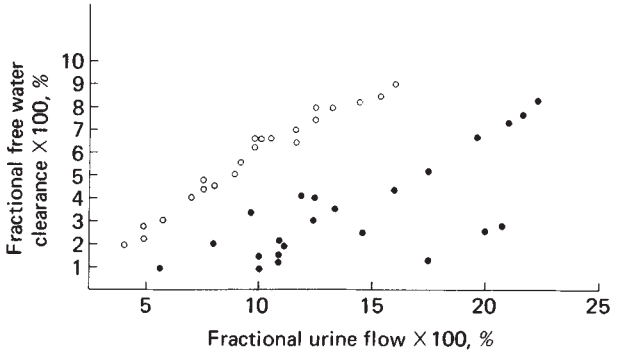

Fig. 2. *The relationship between fractional free water clearance and fractional urine flow in control* (open circles) *and experimental* (closed circles) *animals.*

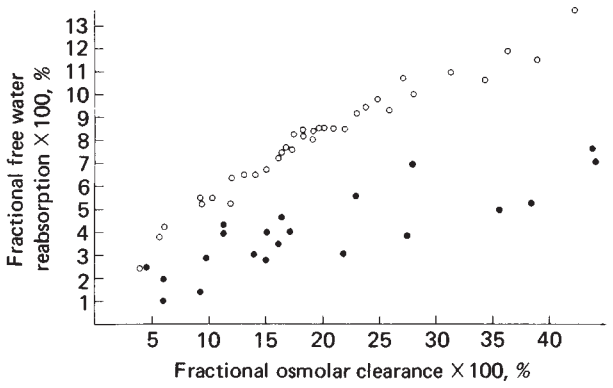

Fig. 3. *The relationship between fractional free water reabsorption and fractional osmolar clearance in control* (open circles) *and experimental* (closed circles) *animals,*

196 administration are in direct contrast to the results obtained following the administration of chlorothiazide, where water reabsorption is inhibited but urate reabsorption remains unchanged [2]. Taken together, the results of these studies suggest that sodium and urate reabsorption are not intimately linked as in a co-transport process.

The urinary precession of urate from inulin was utilized as an index of urate secretion. In control animals, the arrival times of [2-^{14}C]-urate and [methoxy-^{3}H]-inulin and the ratio of counts of urate to inulin in the first urine sample to contain significant counts of [methoxy-^{3}H]-inulin are similar to those previously reported from this laboratory [2]. MK-196 obliterated the differences in the arrival time of the two isotopes, as well as reversing the increase in the ratio of counts seen in controls, indicating inhibition of urate secretion. This suggests that the major portion of the urate which ultimately appears in the urine following the administration of MK-196 is that fraction of filtered urate escaping reabsorption.

In summary, then, MK-196 is a potent uricosuric and diuretic agent in the rat. Following the administration of this agent, in the doses employed, both urate reabsorption and secretion are inhibited. The resultant effect is a marked increase in the urinary excretion of urate due to inhibition of urate reabsorption in the proximal convoluted tubule. By contrast, the natriuresis which ensues the administration of this agent is due to inhibition of sodium chloride transport at a site distal to the proximal tubule, presumably the ascending limb of the loop of Henle.

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